U.S. Patent Application Serial No. 09/322,353 Attorney Docket No. 9855-26U1 (OTT 3038-1)

Clean Copy of Substitute Paragraphs, As Amended in the Amendment Corresponding to the Office Action Dated 30 January 2001

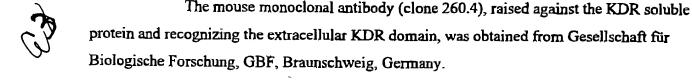
i) Please delete the paragraph at page 4, lines 2-3 and substitute in place thereof the paragraph amended to read as follows.

In a further aspect, the KDR⁺ cells are isolated using a conjugated vascular endothelial growth factor or a molecule derived therefrom.

ii) Please delete the paragraph at page 14, lines 21-28 and substitute in place thereof the paragraph amended to read as follows.

The invention also includes a method of obtaining a cell population enriched for long-term repopulating human hematopoietic stem cells wherein KDR⁺ cells are isolated using a conjugated vascular endothelial growth factor. This method simply capitalizes on the affinity of the KDR-VEGF receptor-ligand interaction to select cells expressing KDR on their surfaces by binding such cells, via the KDR present on the surface of the cell, to VEGF conjugated to, for example, a solid support matrix. Thus, the VEGF-conjugate can be used to affinity-purify the KDR expressing cells by standard methods well-known in the art.

iii) Please delete the paragraph at page 40, lines 16-18 and substitute in place thereof the paragraph amended to read as follows.







(c)

iv) Please delete the paragraph at page 42, lines 1-1/4 and substitute in place thereof the paragraph amended to read as follows.

HPCs were seeded in 0.9% methylcellulose fetal calf serum free (FCS') medium supplemented with saturating amounts of HGFs [flt3, kit ligand (FL, KL), basic fibroblast GF (bFGF) (100 ng/ml each), interleukin 6 (10 ng), IL3 (100 U), granulomonocyte colony-stimulating factor (GM-CSF) (10 ng), G-CSF (500 U), M-CSF (250 U), thrombopoietin (Tpo) (100 ng), erythropoietin (Epo) (3 U)]. CFU-Mix/BFU-E and CFU-GM colonies comprised >5 x 103 and >10³ cells, respectively (Gabbianelli et al., 1995, Blood 86:1661-1670). A more limited HGF combination comprised IL3, GM-CSF, Epo at the indicated dosages (Gabbianelli et al., 1995, Blood 86:1661-1670) (this culture condition was also utilized for NOD-SCID mice BM mononuclear cell (MC) clonogenic assay). CFU-Mix/BFU-E and CFU-GM colonies comprised >500 and >100 cells respectively. For detection of human colonies, the colony DNA was processed for PCR using KlenTaq-1 DNA polymerase (Clontech, Palo Alto, CA) and primers recognizing human a-satellite sequences on chromosome 17 (Warburton et al., 1991, Genomics 11:324-333).

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